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PATENT APPLICATION

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In re Application of:

Thomas S.Y. KO et al Conf. No.: **5596**

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Filed: **January 25, 2002** Examiner: **Unknown**

For: **METHOD OF PREPARING BIOLOGICAL MATERIALS AND PREPARATIONS PRODUCED USING SAME**

SUBMISSION OF PRIORITY DOCUMENT

Commissioner for Patents
Washington, D.C. 20231

Sir:

Submitted herewith is a certified copy of the priority document (i.e., Australia PR2729)on which a claim to priority was made under 35 U.S.C. § 119. The Examiner is respectfully requested to acknowledge receipt of said priority document.

Respectfully submitted,

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AUSTRALIA

Patent Office
Canberra

I, LEANNE MYNOTT, MANAGER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. PR 2729 for a patent by THOMAS KO SAI-YING and TERENCE PING YUEN AUYEUNG filed on 25 January 2001.

I further certify that the above application is now proceeding in the name of GAINFUL PLAN LIMITED pursuant to the provisions of Section 113 of the Patents Act 1990.

WITNESS my hand this
Thirtieth day of January 2002

7

LEANNE MYNOTT
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Our Ref: 7538470

P/00/009
Regulation 3:2

AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

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SEC 113

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Invention Title: **Method of preparing biological materials and preparations produced using same**

The invention is described in the following statement:

METHOD OF PREPARING BIOLOGICAL MATERIALS AND PREPARATIONS PRODUCED USING SAME

FIELD

The present invention generally relates to a method of preparing biological materials, and particularly, but not exclusively, to a method of preparing biological proteins.

BACKGROUND

Many biological materials, such as proteins or whole cells, which may be useful in treatment and prevention of human and animal diseases or as food supplements, for example, are known to have a limited shelf life. This limitation is generally considered to be a result of protein instability at storage temperature, for example room temperature. The shelf life of certain proteins, and/or, cell cultures, may be extended by storing them at refrigeration temperatures (ie, 4-8°C), however, even at such temperatures a shelf life of less than 18 months is common.

As will be appreciated, biologically active proteins are generally folded in a complex three dimensional manner which is unique to each protein. The proteins are generally organised on three levels; having a primary structure, consisting of a linear chain of covalently bonded amino acid residues (a peptide chain); a secondary structure, in which the peptide chain folds into regular patterns (such as, alpha helices and beta-pleated sheets); and a tertiary structure in which the folded chain further folds upon itself to form a compact structure. In addition, some proteins consist of more than one polypeptide chain held in close arrangement to form what is referred to as the quaternary structure. It is the tertiary and/or the quarternary structure which dictates a proteins ultimate biological activity.

The ultimate structure of a protein may be effected by a number of environmental factors; for example, temperature, pH, the presence or absence of certain co-factors or metals, presence of oxygen, enzymes, oxidising or reducing agents and the presence of water or moisture. Where conditions are not optimal, a protein may not form properly or may denature, such that its biological function is lost, or is at least diminished.

The cells of animals, plants and microorganisms may be considered complex protein materials in the broadest sense as they contain numerous proteins enclosed by a cell membrane and/or cell wall, which membrane or wall inturn presents additional proteins at the cell's surface. As with proteins, the viability of a cell is dependent on the environment in which it resides; for example, temperature, pH, the presence or absence of certain co-factors or metals, presence or absence of certain nutrients, metabolic waste, oxygen, enzymes, oxidising or reducing agents and the presence of water or degree of moisture may individually or collectively act to effect viability. As an example, the bacteria *Lactobacilli* and *Bifidus*, which are of commercial significance due to their common usage in yogurt or as a probiotic in human or animal health nutritional products, generally can survive at 4°C for only a short period of time. At temperatures elevated above 4°C, or during heat/freeze-drying, for example, such bacteria die due to dehydration. The main cause of the death of the bacterial cells is thought to be attributed to the denaturation of the proteins residing within the cell and at the cells surface.

Cell cultures (including bacterial cell cultures) and biological proteins are normally made in solution. However, water is known to hydrolyse protein in a time and temperature dependent manner resulting in denaturation and potential loss of function. Dehydrating such cultures or protein solutions may not improve their stability as during dehydration, and at the high temperatures at which known dehydration procedures may occur, the proteins may also be denatured. Refrigeration of cell cultures and proteinaceous solutions, or the freeze-drying thereof, has been used in an attempt to curb such problems.

Freeze-drying under vacuum (lyophilization) is commonly used in industry to prepare proteins for use in vaccines and the like. The process traditionally involves freezing a solution of the biological protein removing ice crystals therefrom by converting them into water vapor under vacuum (sublimation). Unfortunately, this process can cause damage to the native structure of the protein.

To help increase the stability of a biological protein being prepared by freeze-drying, additives such as buffering or stabilising agents may be used in the product formulation. However, during freeze-drying, when the temperature of the solution is slowly reduced to minus 20°C over a period of days, the additives may solidify at different freezing points. As a result, the end product may be a fine puffy cake-like substance actually made up of different layers, each representing an individual component. In essence, the additives added to protect the biological protein may be physically and chemically separated therefrom rendering them useless as protective agents.

An alternative procedure, which is commonly used in the food and dairy industry, to make dry fruit concentrates and milk powders, for example, is spray-drying-using-heat. This process involves spraying a fine mist of solution downwards from the top of a spray tower against an upward current of hot air. The hot air removes water from the droplets before they reach the bottom of the tower. Spray drying normally operates at an inlet air temperature exceeding 190°C and the product temperature may well exceed 60°C. At this operating environment, most of the biological protein or cells, such as bacterial cells, denature.

Fluid bed spray drying is a modified spray-drying-using-heat technology. The process is commonly used in the pharmaceutical and chemical industry for tablet granulation and/or for drying heat stable materials. The process involves spraying a fine mist of solution containing actives downward from the top of a spray head towards a mass of dry excipients. Simultaneously, an upward current of hot air is passed through the mass of excipients to create a fluidised bed. The hot air removes water from the fluidised wet solids at the bottom of the fluid bed.

Fluid bed spray drying technology may be applicable to pharmaceutical proteins which are heat stable around 50-60°C. However, the native structure of the protein may be compromised and accordingly the protein may loose all, or at least some, of its biological activity.

Further problems may be associated with fluid bed spray drying as described above; for example, the spray nozzles, which are positioned near the top of the processing chamber, are required to have substantial clearance above the surface of the fluidised bed of excipient materials so that the such materials do not block the spraying nozzles; a substantial amount of the coating material, or liquid containing the active ingredient(s), may block the nozzles' filter system leading to processing loss; and such top spraying fluid bed operation may only be ideal for granulation rather than for spray coating purposes.

Fluid bed spray drying apparatus have been designed which spray liquid containing the active ingredient(s) from the bottom of the processing chamber. For example, the Roto-processor™ (Aeromatic, Switzerland) designed for pellet coating (pellets of approximately 1mm or above in diameter), and the Aerocoater™ processor (Aeromatic, Switzerland) designed for coating kernels, granules, pellets and small tablets. It is considered that neither the Roto-processor™ nor the Aerocoater™ are designed for micro-particle coating.

Of the techniques available, prior to the development of the present invention, for preparing biological proteins and cells, the technique of micro-encapsulation may be considered the most useful. Typically, no major equipment is required and the batch size can be as small as 10-20g thus making it useful for the preparation of biological proteins that may not be plentiful. This process uses organic solvents to solubilise the biological protein which is then encapsulated in polymeric microspheres using either a water-in-oil-in-water (w/o/w) or a solid-in-oil-in-water (s/o/w) emulsion method. Protein is captured into the solid microspheres after water is removed by simple filtration and the solvent is evaporated off.

Micro-encapsulation technology has been used to make carbon or self-adhesive paper in the paper industry and at least in Japan, food products, such as artificial fish eggs and decorative products are made using gelatin microcapsules to entrap fish or meat flavours.

While micro-encapsulation may be considered a favourable means to prepare biological proteins and whole cells for storage and future use, the technology is still at the

developmental stage in the pharmaceutical and biotechnological industries. The technology has apparent difficulties in that proteins are likely to be denatured by the solvents used and by the necessary emulsifying/homogenising process. In addition, the quality of a product produced according to this process, may be considered undesirable due to the fact that traces of solvent remain in the core of the microcapsules; the traces of such solvents may hamper the commercialisation of a product produced using this technology.

If biologically active proteins and viable cell cultures could be prepared such that they were substantially stabile at room temperature, it may increase their shelf life and obviate the need for refrigeration. At the same time, various alternative drug delivery methods could be explored, such as conventional oral delivery, sublingual delivery, nasal delivery, buccal delivery, occal and even dermal delivery. Such alternative administration methods may minimize the invasive nature of the commonly used injection delivery, and create vast commercial opportunities to fully explore the use of all these molecules.

Bibliographic details of the publications referred to herein are collected at the end of the description.

OBJECT

It is an object of the present invention to provide an improved method of preparing biological materials, and biological materials produced therefrom, or at least to provide the public with a useful choice.

STATEMENT OF INVENTION

In one broad aspect of the present invention there is provided a method of preparing products containing moisture-sensitive materials, including biological materials such as proteins, peptides or live cells, comprising at least the steps:

(i) providing a coating liquid comprising at least one active, a sugar polymer and a water soluble/miscible solvent;

(ii) providing a quantity of micro-particles comprising at least water soluble gel forming solid particles;

(iii) fluidising said quantity of micro-particles within a processing chamber of a of a suitable apparatus to form a fluidised bed of said micro-particles;

(iv) spraying said coating liquid onto said fluidised bed from beneath the fluidised bed to coat said micro-particles therewith under saturated moisture conditions; and

(v) allowing coated micro-particles to dry.

The process of the invention may further comprise one or more additional coating steps which further coat the micro-particles with an enteric coating, a film coating, a moisture repellant coating or taste masking coating.

Preferably the coated micro-particles are heat dried.

Preferably, said active comprises proteins, peptides, or cells.

The coating liquid of the present invention preferably comprises additional constituents such as amino acids, proteins, chelating agents, buffers, preservatives, stabilisers, antioxidents, lubricants and other additives which may act to compliment the function of, or stabilise, the active contained therein.

Preferably said water soluble/miscible solvent is either or both of glycerol or propylene glycol.

Preferably said sugar polymer is selected from one of the following; dextran, fructose, fruitose, glucose, invert sugar, lactitol, lactose, maltitol, maltodextrin, maltose, mannitol, sorbitol, sucrose, trehalose or combination thereof.

Preferably said water soluble gel forming solid particles comprise at least one or more of the following; acrylate and derivatives, albumin, alginates, carbomers, carrageenan, cellulose and derivatives, dextran, dextrin, gelatin, polyvinylpyrrolidone, and starch.

Preferably binding agents selected from one of the following polymers of acrylate and derivatives, albumin, alginates, carbomers, carrageenan, cellulose and derivatives, dextran, dextrin, gelatin, polyvinylpyrrolidone, starch or combination thereof.

Preferably the process is conducted in a Huttlin Turbojet™ Coater.

Preferably the product processing weight exceeds 50% w/v of the fluid bed processing chamber. More preferably the processing weight exceeds 75% w/v.

Preferably the process is conducted in a moisture saturated environment.

Preferably the process is conducted within the processing chamber of the apparatus in an enclosed sterile environment.

Preferably the process is conducted in an oxygen-free environment. In such case, the air within the processing chamber may be replaced by nitrogen, or another suitable inert gas.

A room temperature stable product produced according to the method herein described.

Preferably, said product contains at least one of a protein, peptide or a cell.

Preferably said product is suitable for use in a composition for injection, as sublingual tablets, oral tablets, sustained release sublingual tablets, microcapsules, feed premix, pessaries, pre constituted solid dose for nasal spray or drops, aqueous drops, eye wash or drops, or a skin washing solution.

A method as herein described when used to stabilise biological materials.

A method for creating stable sustained release tablets or microcapsules for ingestion by an animal, including a human.

A method for creating a tablet or microcapsules to be administered to an animal, including a human, said tablet having a protective enteric coating.

A method of producing a substantially room temperature stable anti-diarrhoea agent as herein described.

A method of producing a substantially room temperature stable growth promotant formulation as herein described.

A method of producing a substantially room temperature stable weight loss agent as herein described.

A method of producing a substantially room temperature stable tablet or microcapsules containing beta-1,3-glucan as herein described.

A method of producing a substantially room temperature stable product containing interferon as herein described.

A method of producing a substantially room temperature stable product containing *Bifidus*.

A method of producing a substantially room temperature stable product containing *Lactobacilli*.

A method of producing a substantially room temperature stable product containing *Lactobacilli* and *Bifidus*.

A method of producing a substantially room temperature stable product containing alternative probiotics or micro organisms.

Substantially room temperature stable products produced by the method described herein.

The invention may also be said broadly to consist in the parts, elements and features referred to or indicated in the specification of the application, individually or collectively, in any or all combinations of two or more of said parts, elements or features, and where specific integers are mentioned herein which have known equivalents in the art to which the invention relates, such known equivalents are deemed to be incorporated herein as if individually set forth.

PREFERRED EMBODIMENT(S)

These and other aspects of the present invention, which should be considered in all its novel aspects, will become apparent from the following description, which is given by way of example only. It will be appreciated that, while not explicitly mentioned herein, a number of modifications may be made to the invention without departing from the scope thereof.

Background

During tablet manufacturing using fluid bed technology the present inventors discovered that the tablets produced were always free of bacteria even when the raw materials had bacterial counts in excess of 1000CFU/g (CFU = colony forming units). In order to clarify which of the processing parameters was responsible for the apparent bactericidal effect, the inventors designed an experimental protocol which is described in general terms below.

The experiment was conducted in a Huttlin Turbojet Fluid Bed Coater (BWI Huttlin, Daimlerstrasse 7, D-79585, Steinen, Germany) with a 5L processing container fitted with three bottom spray 3-component spray nozzle jets and standard 20 micron filter bags. Samples of *Lactobacilli* were sourced from Chr. Hansen of 49 Barry Street, Bayswaster, Melbourne, Australia. *Lactobacilli* with *Bifidus* at a ratio of 1:1 was sourced from Gist-brocade, Australia. These bacteria are anaerobes which easily perish in the presence of oxygen. The trial batch size of the fluidised bed was 4 kg, twice the weight recommended for the 5L Huttlin Turbojet processing container used, to ensure the fluidised material was close to the processing containers filter giving the best chance for the bacteria to escape through the 20 micron filter. The solid core (tablet granule core materials) comprised

66%w/w dextrose, 13%w/w gelatin, 15%w/w starch. The spraying liquid containing bacteria comprised either 1.16 x 10^{12} CFU *Lactobacilli*, or 3.5 x10^{12} CFU *Lactobacilli/Bifidus*, with 3%w/w mannitol, 1%w/w albumin, 1%w/w glycerol, 1%w/w sodium phosphate buffers and made up to 1000ml with purified water.

The solid core material was loaded into the Huttlin Turbojet by vacuum and fluidised at a rate of 250 or 300 cubic meter of air/hour. Subsequently, the spray liquid was sprayed into the processing container at a rate of 30 gram/min. The process was conducted at a product temperature of 40–45°C. The material was dried to less than 5% moisture content at a product temperature of 40°C.

It was discovered that running the experiment with a fluidisation rate set at 250 cubic meters of air/hour was not sufficient. At this rate the fluidised material crashed when 95% of the liquid was sprayed into the solid core. Fluidisation at a rate of 300 cubic meters of air/hour overcame this problem.

Samples of the granules obtained during this process were retained and the rest of the granules were compressed into tablets after blended with a standard tablet lubricant. The granules and the tablets were analysed to assess their live bacterial count. The results obtained are provided in Table 1 below.

Table 1

Sample Description	Theoretical activity in CFU/4kg time zero	Reported activity in CFU/4kg time zero	Reported activity in CFU/4kg sample kept at 4°C for 60 days
Chr. Hansen Granules	1.16×10^{12}	1.70×10^{11}	1.64×10^{11}
Chr. Hansen Tablet	1.12×10^{12}	4.40×10^{11}	4×10^{10}
Gist-Bocade Granules	3.52×10^{12}	9.80×10^{10}	4.80×10^{10} (Lactobacilli only) (No Bifidus detected)
Gist-Bocade Tablet	3.44×10^{12}	6.00×10^{10}	1.2×10^{10} (Lactobacilli only) (No Bifidus detected)

It was extremely surprising to detect the presence of viable bacterial cells in the samples after the treatment the bacterial cells were exposed to in the processing of the granules and tablets; heating, possible total ventilation through the 20 micron filter, mechanical milling, air drying and tablet compression. That a substantially high number of bacteria survived during these experiments did not elucidate why the tablets the inventors had previously produced were bacteria free. The results did, on the other hand, suggest a novel process to prepare samples or products containing live bacteria in a stabilised form.

The suppliers of the *lactobacilli* and *bifidus* cultures indicated that these bacteria are extremely temperature sensitive, and heat labile. When such cultures are processed using freeze-drying, bacterial counts are noted to drop by 90 – 99%. Further, the suppliers have indicated that the bacteria are also particularly sensitive to standard tablet compression processes and accordingly that a further drop in activity of the bacteria of approximately 99% may be observed.

Accordingly, the inventors have discovered a novel heat drying method which appears to be superior to known industrial methods of preparation, such as freeze-drying. The

inventors have further found that bacteria processed in this novel manner (ie in micro-capsules) can survive tablet compression pressure around 5 to 8 tons/square inch.

General description

The present invention provides a novel way of drying and preserving moisture-sensitive materials, particularly biological materials such as proteins, peptides and plant and animal cells, including micro-organisms. In general terms, the method combines the technologies of fluid bed spray processing and micro-encapsulation.

In general terms, the process involves the spraying of a liquid containing at least an active of interest, in combination with at least one sugar polymer, and a water miscible/soluble solvent, onto an acceptable particulate excipient material (micro-particles) which is appropriately fluidised in a processing chamber, at temperatures elevated above room temperature. The coating of said micro-particles provides for the stable micro-encapsulation of the active ingredient.

As used herein an "active" generally includes proteins, peptides and cells. However, those of skill in the art to which the invention relates will readily appreciate other materials or active agents which may benefit from preparation according to the invention. It will be appreciated that as used herein the term "proteins", "peptides" and "cells" refer to those which have been produced artificially in the laboratory, via chemical synthesis, or recombinant techniques, in addition to those which are naturally occurring.

The micro-particles comprise water soluble gel forming solid particles, preferably having an adhesive surface, consisting of either natural or synthetic polymers or monomers which can tolerate relative high moisture content without turning into liquid or semi solids. For example, the micro-particles preferably comprise at least one of acrylate and derivatives, albumin, alginates, carbomers, carrageenan, cellulose and derivatives, dextran, dextrin, gelatin, polyvinylpyrrolidone, starch or a combination thereof. Preferably, the micro-particles comprise albumin, gelatin, and pregel starch. The micro-particles are preferably 100 microns in diameter, however, it will be appreciated that alternative sizes may be

utilised in the invention; for example 50 micron to 1mm particle size. It should be noted that the water soluble gel forming solid particles of the micro-particles, may be referred to herein as a "hydrogel core".

It will be appreciated that the composition of the micro-particles used in the invention ensures the liquid spray material containing the active binds efficiently to the surface of the micro-particles without agglomeration or loss.

During processing the micro-particles are required to be saturated with moisture to ensure the surface of the particles are not overheated and a thin film is formed on the surface thereof. The composition also has the advantage that it dries in a comparatively slower manner than hard surface particles and also in a continuous manner to give a complete surface coating.

As described briefly above, the spray or coating liquid comprises at least an active, a sugar polymer and a water soluble/miscible solvent. The sugar polymer is preferably mannitol or dextran. However, it will be appreciated that alternative polymers may be used depending on the precise nature of the active contained within the solution. Suitable sugar polymers may include, for example, fructose, fruitose, glucose, invert sugar, lactitol, lactose, maltitol, maltose, maltodextrin, sorbitol, sucrose, trehalose, or combinations thereof. The water soluble/miscible solvent is preferably either or both of glycerol or propylene glycol; however, those of general skill in the art to which the invention relates may realize alternative solvents suitable for use in the invention.

The spray or coating liquid may also contain additional constituents such as further proteins, amino acids, diluents, chelating agents, buffers, preservatives, stabilisers, antioxidants, lubricants and other additives which may act to compliment the function of, or stabilise, the particular active contained therein. The precise nature of such additional constituents, will depend on the nature of the active. However, examples each include: amino acids, lysine, glycine, L leucine, isoleucine, arginine, cysteine; proteins, human serum proteins, albumin, egg albumin, gelatin; buffers, various sodium phosphate buffers,

citric/citrate buffers, tris buffer; preservatives, derivatives of hydroxybenzoic acids; antioxidants, vitamin E, ascorbic acid; lubricants, water miscible silicone/silicates; chelating agents, citric acid, EDTA, EGTA. Those of skill in the art will appreciate a variety of other proteins, amino acids, diluents, chelating agents, buffers, preservatives, stabilisers, antioxidants and lubricants which may be suitable for use in the present invention.

The process is preferably conducted in an enclosed sterile environment. As used herein a "sterile environment" is taken to be an environment which is substantially free of contaminating material. Generally such "contaminating material" will comprise micro-organisms or the like however, those skilled in the art will appreciate other materials which may be desired not to be present during processing of a product.

The environment in which the process is conducted is preferably free of oxygen to minimise oxidation of actives, for example. This may be achieved by replacing the air contained within the processing chamber, in which the processing of the method of the invention substantially takes place, with an inert gas, preferably nitrogen. However, it will be appreciated that alternative gases may be utilised, such as carbon dioxide.

As briefly mentioned herein before, the micro-particles are required to be saturated with moisture. Accordingly, the process is said to be conducted in a moisture saturated environment. In a "moisture saturated environment" the conditions are such that the surface of the micro-particles will begin to dissolve changing from a totally solid state to a substantially liquid state. Moisture saturation is achieved in the present invention by over spraying the coating liquid onto the hydrogel core.

The process of the invention may be carried out in any appropriate fluid bed spraying apparatus. In the Examples elucidated herein a CPU Driven Turbojet™ Fluid Bed Coater, manufactured by BWI Huttlin (Daimlerstrasse 7, D-79585, Steinen, Germany) has been used. Those of general skill in the art to which the invention relates will be familiar with

such apparatus. However, further information may be readily obtained from the manufacturer if necessary.

It will be appreciated that modifications may be made to the apparatus used in the process of the invention in order to facilitate efficient and effective micro-encapsulation. For example, the Huttlin Turbojet used in the examples described herein, was custom modified as follows:

- The spray nozzle was redesigned such that the centre part of the nozzle (which delivers the liquid spray to the processing chamber) may be removed during operation of the apparatus, for cleaning or unblocking the nozzles. This modification allows for continuous processing.
- The central air return column present in the standard Huttlin Turbojet apparatus was rearranged and replaced with a cone-like arrangement such that at high velocity, the fluidised material moves in a vortex-like manner, and at low velocity, circulates in a whirlpool motion. It is considered that such a modification allows for improved coating of the micro particles.
- All contact surfaces were extra mirror polished such that they can be readily heat sterilised after the standard Cleaning-In-Place cycle.
- Additional compressed air spray nozzles facing the inner surface of the processing container/chamber wall were added surrounding the existing dynamic filter system arrangement. This modification may provide a continuos stream of compressed air flowing from the top of the chamber along the surface of the inner chamber ensuring the working surface in the process container is cleaned continuously. This modification ensures the equipment can be operated continuously without repeat cleaning.
- The processing air is so designed that at any stage a recirculating inert gas, such as nitrogen, can be introduced for fluidisation instead of air. This modification may reduce biological protein oxidation and increase anaerobic bacteria stability.

The micro-encapsulation process of the invention uses an unconventional bottom spray coating operation. That is spraying of the spray or coating liquid occurs from the bottom of the processing container upwards. As such, it will be appreciated that the spray nozzles are

actually embedded within the fluidised bed. Depending on batch size there can be up to 38 spraying nozzles operating at the same time.

The spray liquid is processed in such manner that it transforms itself into a continuous glassy film ("bioglass" film) wrapped around the solid surface of the fluid bed particles. The transformation from liquid to glassy solid is rapid, preventing denaturing of the biological protein or micro-organism. The active, such as a biological proteins or micro organisms do not suffer in the heat, which is dissipated by the latent heat of evaporation of water.

The process of the present invention preferably involves the over weighting of the micro-particles into the processing chamber. In normal fluid bed operation it is recommended by equipment manufacturers not to exceed 50%w/v capacity of the processing chamber. For example, if the processing container is 100 L, processing material weight should not be more than 50kg. However, the process of the present invention allows for (and it is preferable to do so) the processing weight:container volume to be more than 50% w/v. In this manner, the weight of the micro-particles may act like a sieve so that when the encapsulation process is initiated, the spray liquid will not pass through the fluidised micro-particles and out through the dynamic filter system resident at the top of the processing chamber.

The method of the invention may be described in general terms as follows:

1. Solid hydrogel particles (micro-particles) of a suitable constitution are loaded into the Turbojet by vacuum and fluidised. Fluidisation may occur at a rate of between 200 to 500 cubic meters per hour.
2. The micro-particles are preferably heated to 30°-80°C, more preferably to 60°C, for approximately one hour with high velocity processing air so they are fluidised in a vortex-like motion, ensuring that the inner part of the micro-particles are dry.
3. Micro-particle temperature is preferably reduced to 35°C–55°C and the processing air velocity is similarly preferably reduced so that the micro-particles move in a whirlpool-type manner.

4. When the micro-particle temperature reaches preferably approximately 40°C-50°C, the processing air is preferably replaced with an inert gas, such as nitrogen. This step is preferably held for at least approximately 10 minutes to ensure all the air is replaced with nitrogen.
5. Active is immobilised in an appropriate spray or coating solution. The base solution is preferably heated to 38°C to allow complete solid dissolution. Prior to Turbojet spray coating the biological materials are added to the base solution (mixing at approximately 60rpm) and mixed well.
6. A desired quantity of coating solution is then Turbojet Spray Coated onto the fluidised micro-particles preferably at high speed (preferably at the highest available speed) so the micro-particles are saturated with moisture but still able to freely flow in a whirlpool manner. Spray coating preferably takes place at a rate of 30 gram to 60 gram per minute. The spraying of said coating solution or liquid onto the micro-particles occurs from beneath the fluidised bed.
7. Turbojet coating speed is slowed to preferably between 10 to 20 gram per minute when the micro-particles are saturated with moisture, to ensure the bed of micro-particles is continuously flowing in a whirlpool manner. In this manner the coating solution containing the biological protein is continuously dehydrated in a moisture free nitrogen environment, for example. The product is typically dried to result in a water activity of less than 0.25.

It will be appreciated that the above processing steps and parameters may be altered to accommodate the production of various product forms, or products comprising different actives. Alterations may be made for example to: the inlet process air temperature, the product temperature, fluidized air volume, liquid spraying speed, spray liquid temperature, spray liquid viscosity, spray liquid solid content, total core surface area, water solubility of core, humidity of inlet air, compressed air spraying pressure, the apparatus filter pore size, and the frequency of auto dedusting. Where an alteration is made to one parameter, a person of general skill in the art to which the invention relates will readily be able to identify any corresponding adjustments which may be required in another parameter to

compensate for the first said alteration. In addition, by increasing the molecular weight of the hydrogel core sustained release solid dosage can be created.

Further, additional coating steps may be added to the above general process according to the invention, in order to obtain products having desired characteristics. For example, prior to or after the drying step, the resultant product, or microcapsules, may be coated with further coatings. Those of skill in the art to which the invention relates will immediately realize situations where this may be advantageous; for example, where a resultant product is desired to be administered orally, enteric coatings which may protect the product from degradation in the stomach, and/or, those which allow for sustained or slow release of the active therefrom may be utilized. Generally such further coating will be carried out at a similar coating rate as that used for coating the micro-particles with the initial coating liquid.

The batch size for processing may vary according to the volume of the processing chamber of the apparatus used, and whether overloading thereof is required. In the Examples which are described herein, the batch size is typically 4kg. "Batch size" refers to the total solids used in the processing of the product and constitutes solids contained in both the micro-particles, coating solution, and any additional coating solutions used to formulate the product. Accordingly, as used herein percentages of particular constituents are expressed in terms of the percentage of the total batch size.

Various other modifications will become apparent from the Examples provided herein.

The invention is now further elucidated by reference to the following specific non-limiting examples.

EXAMPLES

Example 1 - Stabilization of micro-organisms

Example 1A

8L of live *Bifidus* culture was obtained from an original 3000L liquid culture from Sine Pharmaceutical Co., Ltd. # 905, Xinjinqiao Rd., Pudong, Shanghai, P.R. China. Data supplied from the manufacturer established that 3000L of fermentation liquid contains a total of $3x10^{16}$ CFU (colony forming units) and yields approximately 8.3kg of freeze dry material containing a total of $2.16x10^{14}$ CFU of *Bifidus*; i.e. after freeze drying, there is reduction of approximately 99% live bacteria population.

After arrival in the laboratory a sample of the culture was tested for stability at 4°C. At time zero 2.43×10^{16} CFU/3000L was recorded. After storage for 14 days under 4°C, the count was reduced to 1.5×10^{14} CFU/3000L; i.e. about 1 in 1500 cells survived after two weeks storage at 4°C.

The liquid culture, containing various sugar additives (as described below) was processed according to the invention in the following manner:

1. Solid micro-particle core (hydrogel core) material was loaded into the Huttlin Turbojet by vacuum.
2. The micro-particles were heated to 60°C for one hour.
3. The micro-particle core temperature was reduced to 40-45°C.
4. The process air was replaced with nitrogen and flushed for 10 minutes.
5. Micro-particles fluidised at a rate of 300 cubic metres of air/hour.
6. *Bifidus* coating liquid was turbojet coated onto the hydrogel core particles under saturated moisture conditions at a rate of 30 gram/min.
7. Resultant product dried to less than 0.25 water activity.
8. Samples of coated microcapsules were tested as time zero and after storage at 4°C, 25°C and 40°C.

This process was conducted using 4 different combinations of micro-particle, or hydrogel core, and coating liquid formulations as indicated below.

SINE RX1	**4kg Batch**
Hydrogel Core 1	
66% w/w dextrose	2.64kg
13% w/w gelatin	0.52kg
15% w/w starch	0.60kg
Coating Liquid 1	
1 x 10^{10} CFU Bifidus	
5% Mannitol	0.20kg
1% standard sodium phosphate buffer	0.04kg
purified water to	1.00kg

SINE RX2	**4kg Batch**
Hydrogel Core 2	
61% w/w dextrose	2.44kg
13% w/w gelatin	0.52kg
15% w/w starch	0.60kg
5% w/w albumin	0.20kg
Coating Liquid 2	
1 x 10^{10} CFU Bifidus	
5% Mannitol	0.20kg
1% standard sodium phosphate buffer	0.04kg
purified water to	1.00kg

SINE RX3	**4kg Batch**
Hydrogel core 3	
61% w/w dextrose	2.44kg
13% w/w gelatin	0.52kg
15% w/w starch	0.60kg
5% w/w albumin	0.20kg
Coating Liquid 3	
1 x 10^{10} CFU Bifidus	
5% dextran	0.20kg
1% sodium phosphate buffer	0.04kg
Purified Water to	1.00kg

SINE RX4	**4kg Batch**
Hydrogel core 4	
61% w/w dextrose	2.44kg
13% w/w gelatin	0.52kg
15% w/w starch	0.60kg
5% w/w albumin.	0.20kg
Coating Liquid 4	
1 x 10^{10} CFU Bifidus	
5% albumin	0.20kg
1% sodium phosphate buffer	0.04kg
Purified Water to	1.00kg

Results recorded are listed in Table 2 below and expressed as CFU equivalent to 3000-L original concentration.

Table 2

Formulation Code	Time zero PRE Bio-encapsulation	Time zero POST Bio-encapsulation	4 weeks POST Bio-encapsulation
SINE RX1 at 4°C	1.5×10^{14}	6.00×10^{12}	6.00×10^{13}
SINE RX2 at 4°C	1.5×10^{14}	2.70×10^{12}	3.69×10^{13}
SINE RX3 at 4°C	1.5×10^{14}	2.70×10^{12}	9.90×10^{12}
SINE RX4 at 4°C	1.5×10^{14}	1.41×10^{13}	1.29×10^{12}
Average			2.99×10^{13}
SINE RX1 at 25°C	1.5×10^{14}	6.00×10^{12}	1.38×10^{13}
SINE RX2 at 25°C	1.5×10^{14}	2.70×10^{12}	2.04×10^{13}
SINE RX3 at 25°C	1.5×10^{14}	2.70×10^{12}	6.30×10^{11}
SINE RX4 at 25°C	1.5×10^{14}	1.41×10^{13}	6.00×10^{12}
Average			1.02×10^{13}
SINE RX1 at 40°C	1.5×10^{14}	6.00×10^{12}	< 10000
SINE RX2 at 40°C	1.5×10^{14}	2.70×10^{12}	4.5×10^{11}
SINE RX3 at 40°C	1.5×10^{14}	2.70×10^{12}	< 10000
SINE RX4 at 40°C	1.5×10^{14}	1.41×10^{13}	< 10000

The results indicate 10 times more live *Bifidus* survive the processing according to the present invention compared to conventional freeze-drying processes. The reaction referred to as SINE RX2 gave the best stability results.

It was found that during processing of Sine RX1 moisture was not effectively picked up by the Dextrose contained within the hydrogel core. When 5% albumin was added to the hydrogel core formulation (see RX2 to RX4) processing was satisfactory.

Example 1B

Further batches of *Bifidobacterium bifidum* 6-1 were imported from Sine Pharmaceutical Co., Ltd. # 905, Xinjinqiao Rd., Pudong, Shanghai, P.R. China. The culture used in Example 1A was thought to contain some waste material which may contribute to instability of the bacteria in the final bio-encapsulated solid micro capsules. Accordingly,

the culture used in the present example had all waste material removed, was concentrated and resuspended in buffer solutions. The culture was assayed on arrival from the manufacturer and a sample was also assayed just prior to use. Results indicated a bacterial count of 4.1×10^8CFU/L.

SINE RX6	**4kg Batch**
<u>Hydrogel core 6</u>	
Pregel Starch 77.5%	3.10kg
Albumin 10%.	0.40kg
<u>Coating Liquid 6</u>	
Bifidus 8.2×10^8 CFU	
Mannitol 2.5%	0.10kg
Glycerol 7.5%	0.30kg
Sodium Alginate 1.5%,	0.06kg
standard sodium phosphate buffer 1%	0.04kg
Purified water to	1.00kg.

SINE RX7	**4kg Batch**
<u>Hydrogel core 7</u>	
Pregel Starch 61%	2.44kg
Gelatin 13%	0.52kg
Starch 15%	0.60kg
Albumin 5%.	0.20kg

Coating Liquid 7

Bifidus 4.2 x 10^8 CFU

Mannitol 1.25%

Glycerol 3.75%

Sodium Alginate 1.5%

standard sodium phosphate buffer 1%

Purified water to	1.00kg

SINE RX8	**4kg Batch**
Hydrogel core 8	
Pregel Starch 63.5%	2.54kg
Gelatin 13%	0.52kg
Starch 15%	0.60kg
Albumin 5%.	0.20kg

Coating Liquid 8

Bifidus 4.2 x 10^8 CFU

Mannitol 0.625%	0.025kg
Glycerol 1.875%	0.075kg
standard sodium phosphate buffer 1%	0.040kg
Purified water to	1.00kg.

SINE RX9	**4kg Batch**
Hydrogel core 9	
Pregel Starch 63%	2.52kg
Gelatin 13%	0.52kg
Starch 15%	0.60kg
Albumin 5%.	0.20kg

Coating Liquid 9

Bifidus 4.2 x 10^8 CFU

Mannitol 0.625%	0.025kg
Glycerol 1.875%	0.075kg
Sodium Alginate 0.5%	0.020kg
standard sodium phosphate buffer 1%	0.040kg
Purified water to	1.00kg.

Processing was carried out according to the protocol used in Example 1A.

Results recorded are listed in Table 3 below and expressed as CFU in 4kg of microcapsules.

Table 3

Formulation Code	Time zero PRE Bio-encapsulation	Time zero POST Bio-encapsulation	2 months POST Bio-encapsulation
SINE RX6 at 4°C	8.4 x 10^8	1.56 X 10^8	2.24 x 10^8
SINE RX7 at 4°C	4.2 x 10^8	5.32 x 10^7	6.00 x 10^7
SINE RX8 at 4°C	4.2 x 10^8	4.72 x 10^7	4.04 x 10^7
SINE RX9 at 4°C	4.2 x 10^8	4.48 x 10^7	2.36 x 10^7
SINE RX6 at 25°C	8.4 x 10^8	1.56 X 10^8	1.64 x 10^8
SINE RX7 at 25°C	4.2 x 10^8	5.32 x 10^7	1.56 x 10^7
SINE RX8 at 25°C	4.2 x 10^8	4.72 x 10^7	2.76 x 10^7
SINE RX9 at 25°C	4.2 x 10^8	4.48 x 10^7	1.40 x 10^7
SINE RX6 at 40°C	8.4 x 10^8	1.56 X 10^8	< 100
SINE RX7 at 40°C	4.2 x 10^8	5.32 x 10^7	< 100
SINE RX8 at 40°C	4.2 x 10^8	4.72 x 10^7	< 100
SINE RX9 at 40°C	4.2 x 10^8	4.48 x 10^7	< 100

It is apparent from the results that the addition of glycerol to the hydrogel core particles further enhances the biological stability of *Bifidus*. It was observed that the addition of alginate to the coating liquid improved fluidisation but did not significantly effect the stability of the bacterium in the final product.

The results obtained from this example again demonstrate that the process of the invention may be considered superior to that of currently used processing techniques, for example freeze-drying; in SINE RX6 it is seen that 1 in 5 bacteria survived processing according to the invention as compared to a reported 1 in 100 in the traditional freeze-drying method.

Example 2 – Stabilization of enzymes

Enzymes are biological proteins which have applications in a variety of industries; for example, they are used in food processing, as animal feed additives, and as human and animal medications.

Example 2a - Stabilized enzymes incorporated into feed as growth promotant

(Enzyme Growth Promotant 2).

Enzymes such as proteases, lipases, amylases, and cellulases, for example, are common additives to animal feed. These enzymes help to increase the bioavailability of the feed.

Animal feed is often manufactured such that the enzymes, together with vitamins and minerals such as copper sulfate and iron, are mixed into the feed. The feed is then generally palletised by steam injection and extrusion. The operating temperature of the feed during palletisation can reach 80°C and above for approximately 10 minutes. Under such conditions many of the enzymes added to the mix may be denatured. In addition, when such feed enters the stomach of an animal, many of the enzymes may be denatured due to the acidic environment therein.

To counter the loss of enzymatic activity due to feed palletisation and the acidic environment in the stomach, current practice is to add massive quantities of enzymes into the feed premix in the hope that at least some of the enzymes will survive.

Accordingly while the use of enzymes in animal feed is theoretically beneficial, the efficacy has not been consistently demonstrated to be economically viable.

Where the added enzymes are sufficiently protected from the harmful environmental factors to which they may be exposed, there may be significant economic and growth benefits. Australian patent application AU07872187 describes a growth promotant comprising microgranules having a core, consisting of one or more immobilised enzymes, encapsulated within a water soluble film and coated with a protective enteric coating. Such a product may help overcome the problems associated with degradation of feed enzymes.

AU07872187 describes a method of producing such a product described in the previous paragraph which typically involves freeze-drying and milling. The present example demonstrates that the method of the present invention may be used to produce an equivalent product, which may significantly reduce the cost of production.

The formulation for the enzyme growth promotant 2 is as follows, expressed in terms of a 4kg batch size:

Hydrogel core

Pregel Starch 67.5%, polyvinylpyrrolidone 10%.

Coating liquid

Protease 2×10^5 Vitapharm Protease Units, Amylase 4.3×10^6 Vitapharm Amylase Units, Lipase 50 Vitapharm Lipase Units, Cellulase 2×10^4 Vitapharm Cellulase Units, Mannitol 2.5%, Glycerol 7.5%, Polyvinylpyrrolidone 1.5%, standard sodium phosphate buffer to pH 7 (1%), Purified water to 1kg.

Enteric Coating Solution (1L batch)

Cellulose Acetate Phthalate10%, Sodium Hydroxide qs to pH 6, Water purified to100%.

Final Acid Rinse Solution

Citric Acid q.s to pH 3, Purified water to 1L.

The growth promotant of the present example is preferably used at a rate of 1kg/ ton feed.

The growth promotant formulation was processed according to the invention using the following protocol:

1. Hydrogel core is vacuum loaded into the Huttlin Turbojet chamber and heated to 60°C for one hour.
2. Hydrogel core product temperature reduced to 45°C.
3. The content of the chamber is fluidised at a rate of 300 cubic metres per hour.
4. Coating liquid turbojet coated onto the hydrogel core under saturated moisture conditions at a rate of 30g/minute.
5. Product dried to less than 5% moisture content.
6. Enteric coating solution turbojet coated onto the core at a rate of 30g per minute.
7. Citric acid solution turbojet coated onto the core at a rate of 30g per minute. The acid acts to reconvert the sodium cellulose acetate phthalate back to cellulose acetate phthalate offering the enzymes enteric protection through the stomach.

It is observed that the process of the invention requires 1/10th of the processing time compared with the previous method of processing described in AU07872187, and also reduces production costs by up to 50%.

In addition, it is noted and was observed that the process does not use any organic solvents or aldehydes, the entire production can be performed in an enclosed environment in one step, exposure of operators to enzymes is greatly reduced and only half the cellulose acetate phthalate is required to offer the same enteric protection.

Furthermore, the process resulted in a product which is stable at room temperature for at least 2 years.

The formulation and process of the present example may be modified by providing an additional final 5% w/w wax coating, such as low melting point macrogol or PEG, for example. In this case, it is believed the microcapsules may be incorporated into a feed mix prior to palletization, with minimal, if any, disruption to enzyme structure; the additional wax coating is able to withstand a short burst of steam and accordingly take up the majority of the heat used during palletization.

Example 2 b. - Stabilized enzymes as weight loss supplement

During studies conducted to determine the appropriate dose rate of the growth promotant 2 described above, it was observed that dosing at 1kg/ton feed gives the optimum feed conversion. However, where the dose rate is increased and reaches 10kg/ton feed, the growth promotant formulation is noted to induce significant weight loss.

Accordingly, a weight loss enzyme supplement was formulated and two open trials were conducted in humans.

The weight loss supplement comprised:

	4kg Batch
<u>Hydrogel core</u>	
Pregel Starch 67.5%	2.70kg
Polyvinylpyrrolidone 10%.	0.40kg

Coating Liquid

Protease $2x10^6$ Vitapharm Protease Units

Amylase $4.3x10^7$ Vitapharm Amylase Units

Lipase 500 Vitapharm Lipase Units

Cellulase 2 X 10^5 Vitapharm Cellulase Units

Mannitol 2.5%	0.10kg
Glycerol 7.5%	0.30kg
Polyvinylpyrrolidone 1.5%	0.06kg
standard sodium phosphate buffers to pH 7 (1%)	0.04kg
Purified water to	1.00kg

Enteric Coating Solution

Cellulose Acetate Phthalate10%	0.40kg
Sodium Hydroxide qs to pH 6	
Water purified to 100%. to	4kg

Final Acid Rinse Solution

Citric Acid q.s to pH 3	
Purified water to	1.00kg

The weight loss supplement was processed according to the invention using the protocol used for the preparation of the enzyme growth promotant 2, described above.

Following processing, the microcapsules were packed in moisture proof sachets, in lots of 1g. A dosage of one sachet mixed in water was taken before each meal.

Example 2b(i) - Weight Loss Study 1

9 volunteers were recruited to determine whether the composition has any weight loss effect, when taken as indicated above.

Results are given in Table 4 below:

Table 4

Subject	Body weight in kg Week 0	Body Weight in kg End of 6 weeks
1	125	116.4
2	107	101.5
3	105	97
4	100	97
5	79	69
6	78	72
7	73.5	66.3
8	73	69
9	68.3	61.5
Total	808.8	746.7
Mean	90	83

A mean weight loss per person of approximately 1.08kg per week, or 7 kg over 6 weeks, was observed.

Weight Loss Study 2

A second study of 20 volunteers was conducted under the supervision of a medical practitioner. Dosage rates were as for "Weight loss study 1".

The results of this study are collected in Table 5 below.

Table 5

Subject	Body weight in kg Week 0	Body Weight in kg End of 3 weeks
1	128	120
2	126	121
3	115	109
4	108	100
5	102	101
6	102	97
7	97	92
8	94	93
9	92	90
10	92	88
11	89	85
12	85	84
13	83	81
14	82	78
15	78	76.5
16	77	70
17	77	70
18	74	71
19	73	72
20	68	60
Total	1842	1758.5
Mean	92.1	88

Mean weight loss per person was approximately 1.37 kg per week, or 4.1 kg over 3 weeks.

The results obtained from the two isolated studies described indicated that the enzyme formulation described herein may be an effective weight loss supplement.

Example 2c - Stabilized bromelaine as anti-diarrhoea medication

It has previously been demonstrated that proteases can be used for treatment of intestinal pathogens in animals, including humans; AU07858587 and AU02367392. Compositions for delivery of such proteases have been described comprising: (i) granules comprising a biologically active material in association with a weak base and partially coated with a delayed release material soluble in intestinal juice; (ii) an acidifying agent having a pH between 1.6 to 6; and (iii) a gel forming agent. The resulted preparations are able to modify the host intestinal surface so that it is not susceptible to bacterial colonisation. Accordingly, the preparation is useful for prevention and treatment of diarrhoea.

The example elucidated below provides an improved method of manufacturing such an anti-diarrhoea formulation.

The anti-diarrhoea formulation comprised:

	4kg Batch
<u>Hydrogel core</u>	
Pregel Starch 72.84%	2.914kg
Polyvinylpyrrolidone 10%	0.40kg
<u>Coating Liquid</u>	
Bromelaine 1.33%	0.053kg
Cysteine1.33%	0.053kg
Mannitol 2.5%	0.10kg
Glycerol 7.5%	0.30kg
Polyvinylpyrrolidone 1.5%	0.06kg
standard sodium phosphate buffers to pH 7 (1%)	0.04kg
Purified water to	1.00kg

Enteric Coating Solution

Cellulose Acetate Phthalate 2.00%	0.08kg
Sodium Hydroxide qs to pH 6	
Water purified to	100%

Final Acid Rinse Solution

Citric Acid q.s to pH 3	
Purified water to	1.00kg

The anti-diarrhoes formulation was processed according to the invention using the following protocol:

1. Hydrogel core loaded into the Huttlin Turbojet processing chamber by vacuum and heated up to 60°C for one hour.
2. Hydrogel core product temperature reduced to 45°C.
3. Hydrogel bed fluidised at a rate of 300 cubic metres per hour.
4. Hydrogel core turbojet coated with coating liquid under saturated moisture conditions at 30g/minute.
5. Product dried to less than 5% moisture content.
6. Enteric coating solution turbojet coated onto core at a rate of 30g/min.
7. Citric acid solution turbojet coated onto the core at a rate of 30g/min; the acid reconverting the sodium cellulose acetate phthalate to cellulose acetate phthalate providing enteric protection for the enzymes within the formulation.

It was observed that this process requires only 1/2th to the processing time compared with conventional methods used to produce such a product. Further, 1/20th of the amount bromelaine is required in the formulation (due to increased stability of bromelaine within the bioglass matrix).

In addition, it is noted that the entire production can be performed in an enclosed environment in one step, exposure of operators to enzymes is greatly reduced and only

$1/5^{th}$ the cellulose acetate phthalate is required to offer the same enteric protection as that gained from the known product described above.

Overall, the production cost is estimated to be reduced to $1/5^{th}$ of that where conventional methods are used to create an equivalent anti-diarrhoea formulation.

Furthermore, it was observed that the process resulted in a product which is stable at room temperature for at least 2 years.

Example 2c(i) - Animal studies involving anti-dairrhoea formulation

A study of the efficacy of the formulation produced according to Example 2c as an anti-diarrhoea treatment was conducted at the Animal Husbandry Research Institute, Jinin Province, China. 90 new born piglets of approximately same weight and age were used in the study.

The piglets were randomly divided into two groups, equal. in sex, weight and age. One group was designated for treatment with a Bromelaine Preparation according to Example 2c and the other half were used as a control group. 0.75g of the Bromelaine Preparation (Example 2c) was mixed with 8.5g of water into a paste on the day of use. A first dose of 10ml was given to the treatment group at day 7 after birth and repeated at day 10. The control group received no treatment. Observation of diarrhoea incidence was recorded up to day 45.

Results are collected in Table 6 below.

Table 6

	Treatment Group	Control
Number of animals	45	45
Birth weight (kg) average	1.40	1.35
Vaccination against diarrhoea	Yes	Yes
Weaning weight (kg) average	11.80	11.30
Total weight gained	10.40	9.95
Daily weight gained	249 g	237 g
Feed consumed	13kg	12.7kg
Feed conversion	1.25	1.29
Pigs that has diarrhoea	3	8
Diarrhoea incidence (%)	6.7	17.8
Animal alive (%)	100	100
Diarrhoea days	3	5
Other medication used	6	10

All piglets used in this trial were vaccinated against diarrhoea. Nonetheless, the treatment group, after two doses of the bromelaine preparation, showed significant weaning weight gain (0.5kg), reduced diarrhoea incidence (6.7 versus 17.8), reduced severity (3 days versus 5 days) and reduced number of other medications used (6 versus 10) compared to control group.

Example 2d - Stabilized enzyme bromelaine plus beta glucan (B&B Preparation) as anti-diarrhoea medication.

Addition of beta-1,3-glucan to the Bromelaine Preparation of Example 2c potentates the anti-diarrhoea action of bromelaine.

Beta-1,3-glucan, is considered an effective natural non-specific immuno-stimulant with free-radical scavenging properties. It is thought to act by activating macrophages, which

play an essential and pivotal role in the initiation and maintenance of the immune response in animals, including humans.

Beta-1,3-glucan is known to be orally effective, completely safe and non-toxic. There are several different types of beta glucan with different levels of activity, the majority of which are inert and used as simple food fillers. Beta-1,3-glucan, is however the most active beta glucan, and may be obtained from the cell wall of yeast.

Beta-1,3-glucan may be considered useful in the treatment of many immune-related indications, such as stress-related immunosuppression, for example, has been shown to act synergistically with antibiotics and antiviral medications and to exhibit antifungal properties. Accordingly, beta-1,3-glucan may be considered a suitable adjuvant for an improved life-style. Those of general skill in the art to which this invention relates will readily be able to identify animal indications which may benefit from the administration of beta-1,3-glucan.

The B&B Preparation of the present example comprises, in the context of an 8kg batch size:

	4kg Batch
<u>Hydrogel core for Bromelaine</u>	
Pregel Starch 72.84%	2.861kg
Polyvinylpyrrolidone 10	0.40kg
<u>Coating Liquid</u>	
Bromelaine 2.66%	0.106kg
Cysteine 2.66%	0.106kg
Mannitol 2.5%	0.100kg
Glycerol 7.5%	0.300kg
Polyvinylpyrrolidone 1.5%	0.060kg
standard sodium phosphate buffer to pH 7 (1%)	0.040kg
Purified water to	1.00kg.

	4kg Batch
Hydrogel core for Beta-1,3-glucan	
Gelatin 72.84%	2.861kg
Albumin 10%.	0.400kg
Coating Liquid	
Beta-1,3-glucan 2.66%	0.106kg
Mannitol 2.5%	0.100kg
Glycerol 7.5%	0.300kg
Polyvinylpyrrolidone 1.5%	0.060kg
standard sodium phosphate buffer to pH 7 (1%)	0.040kg
Purified water to	1.00kg
Enteric Coating Solution	
Cellulose Acetate Phthalate 2.00%	0.16kg
Sodium Hydroxide qs to pH 6	
purified water to 100% (ie to 8kg of total batch size)	
Final Acid Rinse Solution	
Citric Acid q.s to pH 3	
Purified water to	2.00kg.

The bromelain microcapsules were processed according to the invention using the following protocol:

1. Hydrogel core for bromelain loaded into the Huttlin Turbojet chamber by vacuum and heated up to 60°C for one hour.
2. Hydrogel core product temperature reduced to 45°C.
3. Hydrogel bed fluidised at a rate of 300 cubic metres per hour.
4. Coating liquid turbojet coated onto the hydrogel core under saturated moisture conditions at a rate of 30g/minute.

5. Product dried to less than 5% moisture content.
6. Sodium cellulose acetate phthalate solution turbojet coated onto the core product at a rate of 30g/min.
8. Citric acid solution turbojet coated onto the core as a final coat at 30g/min; the acid acts to convert the sodium cellulose acetate phthalate to cellulose acetate phthalate providing enteric protection for the enzymes within the formulation.

The beta-1,3-glucan microcapsules were processed according to the invention using the following protocol:

1. Hydrogel core for beta-1,3 glucan loaded into the Huttlin Turbojet chamber by vacuum and heated up to 60°C for one hour.
2. Hydrogel core product temperature reduced to 45°C.
3. Process air replaced with nitrogen and flushed for 10 minutes.
4. Hydrogel bed fluidised at a rate of 300 cubic metres per hour.
5. Beta glucan coating turbojet coated onto the hydrogel core under saturated moisture conditions at a rate of 30g/minute.
6. Resultant product dried to less than 5% moisture content.
7. Sodium cellulose acetate phthalate solution turbojet coated onto the core product at a rate of 30g/min.
8. Citric acid solution turbojet coated onto the core as a final coat at a rate of 30g/min; the acid acts to convert the sodium cellulose acetate phthalate to cellulose acetate phthalate providing enteric protection for the enzymes within the formulation.

The B&B formulation is then prepared by mixing 4kg of the bromelain microcapsules with 4 kg of the beta-1,3 glucan microcapsules.

Using this protocol, each 750mg of B&B Preparation contains 10mg bromelaine and 10mg beta-1,3-glucan.

In conducting the procedure described in this Example it was noted that the beta-1,3-glucan raw material is a very fine powder with a particle size of less than 5 micron in dry

form. In liquid form, it forms a fine suspension with a particle size les than 2 microns. Accordingly, to fully capture the active ingredients, a more soluble hydrogel based gelatin has to be used.

It was further noted that in most cases a better hydrogel is obtained using gelatin. However, gelatin is comparatively expensive and pregel starch may provide a more economical base for said hydrogel.

A unit dose is prepared by mixing 750mg of B&B Preparation with 8.5g of water to make a 10ml paste.

The recommended dosage for prevention of diarrhoea in piglets is 10ml at day 1 of birth, repeat dosing in day 5. In farms with a serious history of diarrhoea, dosing may be repeated at day 10 & day 13.

Example 2d(i) – Field trials involving the use of B&B preparation

A field trial was conducted in Shangdong, China, to test the efficacy of Bromelaine Plus Beta glucan Preparation in prevention and treatment of diarrhoea in piglets.

B&B Preparation was prepared as in Example 2d. Other medications used in the trial were those standard in the field of animal farming and management.

The trial was conducted at the Breeding Good Pig Farm of Dezhou Husbandry Bureau, which produces over 10,000 pigs annually and which has an incidence of diarrhoea of approximately 40-50%.

Nine litters randomly selected were divided into 3 groups (3 litters/group). Two groups were designated as treatment groups, and the third group as a control. A total of 97 piglets (Large White York piglets of mixed sex) were included in the trial.

Litters were monitored for a period of 26 days from the time of first administration of B&B Preparation.

All piglets in treatment groups and control group were vaccinated and given the same medication when sick (presenting diarrhoea and/or associated symptoms).

The trial design is summarised in Table 7 below.

Table 7

Groups	Number of pigs	Given dose (5 ml)	Appendix
Treatment 1	34	Day 1 one dose, repeat in day 5	Monitor the efficacy of yellow scours
Treatment 2	31	Day 1 one dose, repeat in day 5, 10 and 13.	Monitor the efficacy of yellow and white scours
Control	32	No Bromelaine Preparation given	Normal medications

Results of the trial are summarised in Table 8 below.

Table 8

Groups	# of piglets	Incidence of scour (%)	MDWG (g) (mean daily weight gain)	Mortality	Comments
Treatment 1	34	5.1	164	1	Scour
Treatment 2	31	4.2	178	1	Starvation
Control	32	17.59	169	1	Scour

As is seen from Table 8, the results of the trial demonstrate that the incidence of scours in trial groups are 5.10 %, 4.2% and 17.59 % for treatment group 1, treatment group 2 and the control group respectively. In other words, there was an observed reduction in the

incidence of diarrhoea of approximately 70% between the treatment groups and the control groups. This demonstrated B&B Preparation has a remarkable efficacy in the prevention and/or treatment of diarrhoea in piglets.

No significant difference was observed in mean daily weight gains (MDWC) between groups (164g per day, 178g per day, and 169g per day).

Furthermore, the results suggest that administering the preparation twice provides more efficient improvement of the animal's health.

Overall, this trial indicates that B&B preparation according to the invention has efficacy for pre-weaning scour, and against non-specific *E.coli* diarrhoea. The inventors believe the formulation will have application to other animals, including humans. Those of ordinary skill in the art to which the invention relates will readily be able to modify or adapt the formulation such that it is suitable for administration to animals other than pigs.

Example 3 - Slow Release Sublingual Stabilized Biological

Example 3a - Slow release sublingual stabilized beta-1,3-glucan tablets.

As previously discussed, beta glucans are effective orally. However, when administered orally a substantial dosage is generally required to achieve the desired immuno-modulatory effect. A dosage range of anywhere between 10mg to 2000mg per day has been recommended, depending on the source of beta glucan. The great range in recommended dosages it thought to be due to variation in purity, and bioavailability, of the beta glucan products on the market.

The present example provides a formulation of slow release sublingual beta-1,3-glucan which when properly stabilised (via the process of the present invention, for example) and delivered to a specific mucosal surface, may be clinically active at a dose of 10mg per day.

A product according to the present example may be suitable for the treatment or alleviation of symptoms of an allergic condition, for example hayfever.

An example formula for a slow release sublingual beta-1,3-glucan tablet, processed according to the invention, may comprise the following constituents:

	4kg Batch
Hydrogel core	
Gelatin	2.527kg
Polyvinylpyrrolidone	0.400kg
Albumin	0.400kg
Coating Liquid	
Beta-1,3-glucan	0.200kg
Mannitol	0.200kg
Propylene glycol	0.150kg
Gelatin (succinylated)	0.050kg
standard sodium phosphate buffer to pH 7	0.073kg
Purified water to	2.000kg

A slow release sublingual beta-1,3-glucan tablet of this example was prepared according to the process of the invention as follows:

1. Huttlin Turbojet sterilised using heat (180°C) as instructed by the equipment manufacturer.
2. Hydrogel core loaded into the Huttlin Turbojet chamber by vacuum and heated up to 60°C for one hour .
3. Hydrogel core temperature to reduced to 40°C.
4. Content of chamber fluidised at a rate of 300 cubic metres per hour.
5. Beta-1,3-glucan coating turbojet coated onto the hydrogel core under saturated moisture conditions at 25g/minute.
6. Resultant product dried to less than 3% moisture content.
7. Each 200mg microcapsule contains 10mg of beta-1,3-glucan as measured using the beta glucanase digestion method as described herein after.

8. Product compressed into 200mg tablets according to standard procedures used in the art.
9. Product packed in nitrogen flushed aluminum/aluminum foil pack and stored at a temperature not exceeding 25°C.

The tablets of this example have a slow dissolution rate (>10 minutes) due to the presence of a high percentage of gelatine, polyvinylpyrrolidone and albumin in the hydrogel core. This combination is ideal for slow release products which allow the active material to have continuous contact with the target absorption site, such as the oral mucosal membrane.

The recommended dosage regime for a product according to this example, where it is used for the treatment or alleviation of an allergic condition, is: One tablet dissolved under the tongue daily, for four weeks prior to spring and continue for 6 months thereafter. It is recommended that no food or drink be taken 15 minutes before or after medication.

A product according to this example was trialed in Melbourne, Australia, by a 49 year old female (Mrs Y) and a 39 year old male (Mr. X) both of whom suffered severe hay fever for many years. It was determined using skin sensitivity tests that both subjects suffered from allergic reactions to Rye grass, pollen and house dust.

After taking the tablets of the present example in the dose recommended above, both subjects reported that the incidence of sneezing, itchy eyes and runny nose were minimal this spring season, compared to previous years. Both subjects requested that they be able to repeat the treatment in the following year to determine whether their symptoms may be completely cured.

While not wishing to be bound to any particular theory, the inventors of the present invention believe the sublingual use of beta-1,3-glucan probably desensitised the immune system so that the inflammatory response was down regulated.

The Beta-1,3-glucan sublingual tablet of the present example was also given to one severe asthmatic male aged 40 (Mr. Z) who has to use broncodilator spray and cortico steroid medication consistently. After two weeks of using the medication (one tablet per day, dissolved under the tongue), the wheezing incidence was greatly reduced and the frequency of the need to use the broncodilator spray and cortico steroid medication was halved. However, while the medication was useful to reduce his asthmatic conditions, for some unknown reasons, there were incidences of nose bleeding. The Beta-1,3-glucan medication was stopped accordingly.

Beta Glucanase Digestion Method

The beta glucanase digestion method mentioned previously herein is elucidated in the ensuing paragraphs.

<u>Reagents:</u>

1. Buffer Phosphate (0.06 M , pH 6.0)

5.28g of Disodium hydrogen orthophosphate and 7.29g of sodium dihydrogen orthophosphate is dissolved in 350 ml of distilled water and the pH is adjusted to 6.0 by the addition of 1.0 M phosphoric acid. Adjust the volume to 500ml. Store at 4°C .

2. Yeast Lytic Enzyme

25KU of 81,000 unit/gm Yeast Lytic Enzeme (ICN Biomedicals Inc; cat No 152270) is dissolved in 100ml of Phosphate buffer. Store at -5^{0}C- 4^{0}C. Use within 7 days.

3. Somogyi's Reagent

Dissolve 28g of anhydrous disodium phosphate and 40g of sodium tatrate in 700ml water, add 100ml of 1M sodium hydroxide and stir in 80ml of a 10% solution of crystalline $CuSO_4$, then add 180g anhydrous sodium sulphate , dissolve and make up to a litre. Allow to stand for a few days. Decant or filter. Add the appropriate volume of (M/6N) potassium iodate solution. (see table 8A for volume)

4. 0.005M sodium thiosulphate

Dissolve 1.2409g of sodium thiosulphate in 100ml distilled water. Standardise using method BP 93 to determine the f factor.

5. 2.5% (w/v) Potassium Iodide in water.

6. Fehings solution A: 69.3g $CuSO_4.5\ H_2O$ to Litre.
Fehling solution B : 100g NaOH, 345g $KNaC_4H_4O_6\ 4\ H_2O$ to 1 Litre.
Standardise Fehlings solutions using known glucose standard.

7. Phenolphthalein indicator (1% w/v in 96% ethanol)

8. Methylene Blue 1% aqueous solution

Assay procedure for beta- Glucan

Method 1 (sample weight < 100mg)

1. Weigh 70mg of beta-glucan, add 3ml of buffer solution and 3ml of Lytic Enzyme solution into 10ml volumetric flask.
2. Incubate at 40°C for 24 hours.
3. Cool to room temperature and dilute to 10ml with water.
4. Use same procedure for blank without adding beta glucan.
5. Duplicate samples and blanks have to be carried out at the same time.
6. Filter (centrifuge if necessary) and take 0.5ml for test.
7. Mix 0.5ml of filtrate, 4.5ml of distilled water and 5ml of freshly prepared Somogyi's reagent in a 50ml glass tube.
8. Cover with screw cap and heat in boiling water bath for 10 min.
9. Cool and add appropriate volume of 2.5% potassium Iodide solution from a pipette down the wall of the tube, without stirring or shaking. (see table 8A)
10. Acidify rapidly with 1.5ml of 2N sulphuric acid.
11. Mix and titrate with 0.005M sodium thiosulphate using starch indicator.
12. Carry out a blank on 5ml of water at the same time.

Table 8A

Expected sugar content In 5ml solution sample	**Volume of M/6N potassium iodate to be added**	**Corresponding volume of 2.5% potassium Iodide to be added**
mg	**ml**	**ml**
Up to 0.5	5	0.5
0.5-1.0	10	1.0
1.0-3.0	25	2.0

Method 2 (sample weight >300mg)

1. Weigh 600mg of sample and add 10ml of Lytic Enzyme Solution.
2. Incubate at 40°C for 24 hours.
3. Cool and make up to 100ml with distilled water.
4. Filter in large buchner with 542 paper.
5. Transfer solution to a burette.
6. Add 5.0ml Fehling A and Fehling B to a 300ml Erlenmeyer flask with several anti bumping granules.
7. Heat to boiling and titrate against sugar solution in the burette until the blue almost disappears.
8. Boil solution for 4.5 minutes, add 3 drops of methylene blue solution and continue titration to a brick red endpoint. Endpoint to be reached within 6 minutes of boiling time.

Calculation:

Method 1

% (w/w) 1,3 beta glucan in sample will be calculated as:

$$\frac{(\text{Titre sample} - \text{Titre blank}) \times 0.135 \times f \times 100}{\text{Sample weight used for titration}}$$

f - Factor for 0.005M sodium thiosulphate

Actual concentration = $f \times 0.005$

Method 2

% 1,3 beta glucan in sample will be calculated as:

$$\frac{4.95 \times 100}{\text{Titre sample} \times \text{Sample weight}}$$

Example 4: Stabilization of biologically active proteins

As previously mentioned herein biological proteins and peptides have wide application in a number of industries, including the pharmaceutical industry. However, many of these proteins may be unstable at storage temperatures, such as room temperature.

The efficacy of the present invention in producing a stable protein product is demonstrated in this example using Interferon however, it will be appreciated that it is equally applicable to the preparation of other proteins or peptides.

Interferon alpha, beta and gamma are antiviral, anti tumor and immunity modulating proteins. The method commonly used to introduce exogenous interferon into the body of an animal is by injection. Natural and recombinant interferon alpha 2a and 2b are commercially available as 3 to 10 million IU injections for treatment of viral and tumour diseases. All commercial interferon injection products require storage at approximately 4–8°C because they are unstable at elevated temperatures.

Administration of interfereon by injection, at 3 to 5 million IU dosages, are associated with significant side effects. In addition, as interferon is not a native blood protein, it is quite common that a patient may mount an immune response thereto, after a few injections. Accordingly, subsequent dosages need to be significantly increased in order for the interferon to have effect. This in turn may worsen the side effects. Further, when administered by injection, exogenous interferon will be carried via the blood to the liver and quickly metabolised.

Example 4a - Stabilized Interferon Alpha 3 million IU Injection

The present example provides an interferon injection which is stable at room temperature.

The stabilised interferon injection formulation comprises the following components:

	4kg Batch
Hydrogel core	
Gelatin	3.208kg
Polyvinylpyrrolidone	0.400kg
Coating Liquid	
Interferon alpha 240 billion IU	
Mannitol	0.200kg
Propylene glycol	0.075kg
Gelatin (succinylated)	0.025kg
Glycine	0.012kg
Albumin	0.001kg
Ascorbic Acid	0.006kg
standard sodium phosphate buffers to pH 7	0.073kg
water for injection to	2.000kg

It will be appreciated that the term "water for injection" is one standard in the art. It refers to a standard grade of water suitable for use in formulating injectable compositions, as described in standard pharmacopoea.

The stabilised interferon injection of the present example was prepared according to the invention using the following steps:

1. Interferon alpha, glycine, mannitol, gelatin succinylated, propylene glycol, ascorbic acid and buffers are dissolved in purified water then filtered through 0.02 micron membrane filter. Albumin was added and made up to weight with water for injection.
2. Huttlin Turbojet chamber sterilised using heat (180°C) as instructed by the equipment manufacturer.

3. The Huttlin apparatus was switched to circulating filtered nitrogen mode.
4. Hydrogel core loaded into the Huttlin Turbojet chamber by vacuum and heated up to 60°C for one hour.
5. Hydrogel core product temperature reduced to 40°C.
6. Content of chamber fluidised at a rate of 300 cubic metres per hour.
7. Interferon alpha coating turbojet sprayed onto the hydrogel core under saturated moisture conditions at a rate of 25g/minute.
8. Resultant product dried to less than 2% moisture content.
9. Product packaged in nitrogen flushed injection vials under laminar flow/sterilised conditions.

Each 50mg of the microcapsules prepared according to the present example contains 3 million IU interferon alpha.

Example 4b - Stabilized Interferon Alpha 60,000 IU Pessaries

Room temperature stabilized Interferon pessaries can be made to prevent and treat papaloma infections and other viral diseases in the vaginal surface.

The stabilised interferon pessaries formulation of the present example comprised the following components:

4kg Batch

Hydrogel core

Polyvinylpyrrolidone/Acetic acid ethenyl polymer 3.508kg

Coating Liquid

Interferon alpha 240 million IU	
Mannitol	0.200kg
Propylene glycol	0.150kg
Gelatin (succinylated)	0.050kg
Glycine	0.012kg
Albumin	0.001kg
Ascorbic Acid	0.006kg
standard sodium phosphate buffers to pH 7	0.073kg
Purified water to	2.000kg

The stabilised interferon pessaries of the present example were prepared according to the invention using the following steps:

1. Interferon alpha, glycine, mannitol, gelatin succinylated, propylene glycol, ascorbic acid and buffers were dissolved in water then filtered through 0.02 micron membrane filter. Albumin was added and made up to weight with water for injection.
2. Huttlin Turbojet heat sterilised at 180°C as instructed by the equipment manufacturer.
3. Apparatus switched to circulating filtered nitrogen mode.
4. Hydrogel core loaded into the modified Huttlin Turbojet chamber via vacuum and heated up to 60°C for one hour.
5. Hydrogel core product temperature reduced to 40°C.
6. Contents of the chamber fluidised at a rate of 300 cubic metres per hour.
7. Interferon alpha coating turbojet sprayed onto the hydrogel core under saturated moisture conditions at a rate of 25g/minute.
8. Resultant product dried to less than 2% moisture content.
9. Product compressed into 1g pessaries, according to standard procedures.
10. Product packaged in nitrogen flushed aluminium/aluminium foil packs and stored at a temperature not exceeding 25°C.

Each 1g microcapsule prepared according to this example contains 60,000 IU interferon alpha.

Example 4c - Stabilized Interferon Alpha 2000 IU & Muramidase Sublingual Tablet

Interferon alpha at ultra low dose (less than 10,000 IU/ dose/ adult) has been demonstrated to be an effective medication in treatment of viral and tumour diseases.

Subcutaneously administered interferon alpha is now considered to be the standard therapy for the management of hepatitis B and C and there are a number of commercially available products therefor; for example, Wellferon TM Injection (Glaxo Wellcome), Intron-ATM Injection (Schering Piough), Roferon ATM Injection (Hoffmann-La Roche) and Anferon (Hualida Tianjin, China). However, a number of disadvantages may be associated with the use of such products including, for example: self-administration by patients is painful and requires training; high-doses may be associated with a number of side effects including vomiting, nausea, dizziness, nasal discharge and other flu like symptoms; the cost of treatment may be considered high, for example a normal course of treatment for hepatitis B & C with Roferon A Injection would cost around AU$8000 in Australia; and the products are not stable at room temperature and must be stored under refrigeration.

Studies conducted over approximately the past 10 years have indicated that low doses of interferon alpha may be administered via the oromucosal route (including sublingual administration) with efficacy.

The present example provides a dosage form which may be administered via the oromucosal route (buccal or intra nasal).

The sublingual slow release formulation of the present invention contains both interferon alpha (2000 IU) and muramidase hydrochloride (50mg) and is useful in the treatment or amelioration of chronic viral diseases; muramidase hydrochloride is an antiviral agent with a history of extensive use in Asian countries.

The stabilised interferon alpha & muramidase sublingual tablet formulation of the present example comprised the following components:

	4kg Batch
Hydrogel core	
Gelatin	1.480k
Polyvinylpyrrolidone	0.400kg
Muramidase Hydrochloride	1.000kg
Coating Liquid	
Interferon alpha 2b 40 million IU	
Mannitol	0.200kg
Propylene glycol	0.150kg
Gelatin (succinylated)	0.500kg
Glycine	0.120kg
Albumin	0.020kg
Ascorbic Acid	0.057kg
standard sodium buffers to pH 7	0.073kg
Purified water to	4.000kg

The stabilised interferon alpha & muramidase sublingual tablets of the present example were prepared according to the invention using the following steps:

1. Interferon alpha, glycine, mannitol, gelatin succinylated, propylene glycol, ascorbic acid and buffers were dissolved in water for injection then albumin was added and made up to weight with water for injection.
2. Huttlin Turbojet chamber sterilised by heating at 180°C as instructed by the equipment manufacturer.
3. Apparatus switched to circulating filtered nitrogen mode.
4. Hydrogel core loaded into the modified Huttlin Turbojet chamber by vacuum and heated up to 60°C for one hour.
5. Hydrogel core product temperature reduced to 40°C.
6. Content of apparatus chamber fluidised at a rate of 300 cubic metres per hour.

7. Interferon alpha coating turbojet sprayed onto the hydrogel core under saturated moisture conditions at a rate of 25g/minute.
8. Resultant product dried to less than 2% moisture content.
9. Microcapsules compressed, according to standard procedures used in the art, into 200mg tablets and packed in nitrogen flushed aluminum/aluminum foil packs and stored at a temperature not exceeding 25°C.

Each 200mg tablet produced according to this example contains 2,000 IU interferon alpha 2b and 50mg of muramidase hydrochloride, has a slow dissolution profile and requires not less than 10 minutes to dissolve in the mouth. It is designed as a slow release sublingual product.

A product according to the present example is recommended to be administered for the prevention and/or treatment of chronic viral infections, according to the following preferable dosage regime: One tablet every two days dissolved under the tongue over six months to 1 year.

The stability of the actives (interfereon alpha 2b and muramidase) within the tablets of three different batches of the product produced according to this example was studied at three different temperatures. The results are collected in Table 9 below.

The potency of each active was assessed according to standard procedures used in the art. Briefly, the following steps were taken:

1. The interferon and muramidase were extracted from the existing solid phase into a stable and buffered liquid medium.
2. The liquid extracted was subjected to a Cytopathic Effect Assay (CPE) to determine the antiviral activity of interferon in the tablet (according to the current method of British/European Pharmacopoeia 2000).
3. The liquid extract was subjected to HPLC analysis to determine the quantity of muramidase in the tablets.

Table 9

	IFN+Mu Bx 1	IFN+Mu Bx 2	IFN+Mu Bx 3		IFN+Mu Bx 1	IFN+Mu Bx 2	IFN+Mu Bx 3
	IFN Alpha 2b	IFN Alpha 2b	IFN Alpha 2b		Muramidase HCl.	Muramidase HCl.	Muramidase HCl.
4°C	IU	IU	IU	**4°C**	mg	mg	mg
zero time	2200	1800	2200	zero time	47.5	50.0	47.5
3 months	2400	2200	2200	3 months	47.5	50.0	47.5
6 months	1400	3200	3000	6 months	48.0	48.5	46.0
9 months	2000	2400	1800	9 months	48.0	49.0	47.5
12 months	3000	1600	3550	12 months	46.5	49.0	49.0
24 months	2400	2200	2400	24 months	47.0	48.5	47.5
25°C	**IFN Alpha 2b**	**IFN Alpha 2b**	**IFN Alpha 2b**	**25°C**	**Muramidase HCl.**	**Muramidase HCl.**	**Muramidase HCl.**
zero time	2200	1800	2200	zero time	47.5	50.0	47.5
3 months	2800	2200	2400	3 months	47.5	50.0	47.5
6 months	1000	1400	2850	6 months	48.0	49.0	48.0
9 months	1600	2200	1800	9 months	48.0	49.5	48.5
12 months	2200	1850	2000	12 months	49.0	46.5	48.5
24 months	2400	2200	2200	24 months	48.0	47.5	48.0
35°C	**IFN Alpha 2b**	**IFN Alpha 2b**	**IFN Alpha 2b**	**35°C**	**Muramidase HCl.**	**Muramidase HCl.**	**Muramidase HCl.**
zero time	2200	1800	2200	zero time	47.5	50.0	47.5
3 months	2400	2200	2000	3 months	47.5	50.0	47.5

6 months	2800	2000	2200	6 months	48.5	48.5	48.0
9 months	2000	1600	3800	9 months	48.0	48.5	47.5
12 months	2400	1800	3000	12 months	48.5	48.5	48.0
24 months	2000	2400	2000	24 months	48.0	48.5	48.0

Example 4d - Stabilised Interferon Alpha Nasal Spray for prevention of cold, flu and other respiratory diseases.

Interferon alpha is known to be effective against viral respiratory diseases. Clinical studies in animals, including humans, have demonstrated interferon alpha nasal spray at a dose around a few hundred units to over one million units is effective against respiratory infections, including those associated with the flu and colds. However, at high dosages nose bleeding and flu-like symptoms may be observed.

Correctly formulated interferon alpha 2b in aqueous phase can be stable up to one month, but not more than two months, at room temperature according to the information supplied by manufacturers of interferon alpha 2b. Accordingly, interferon nasal sprays for the treatment and/or prevention of colds, the flu and other respiratory diseases, in a ready to use liquid form, with a viable commercial shelf life (of approximately 12 to 18 months for example) are unavailable.

A viable alternative is to have the principal active ingredient (interferon alpha) presented as a pre-constituted room temperature stable tablet. Accordingly, just prior to use the interferon alpha tablet may be added to a nasal spray bottle containing an acceptable liquid diluent. The reconstituted solution would have a shelf life of around 4 weeks at room temperature; which shelf life would be suitable for the length of treatment of most respiratory infections. The user may discard the bottle at the completion of treatment.

Accordingly, the present example presents a consumer product containing two components:

1. A foiled packed interferon tablet containing 50,000 IU of stabilised interferon alpha; prepared according to the process of the invention; and
2. A bottle containing 5ml of an acceptable diluent, and having a screw on nasal spray applicator.

It is preferred that the nasal spray applicator sprays a metered dose of 0.1ml of solution. According to the present example, at this dosage rate, 1000 IU interferon alpha would be administered with each spray.

A formulation according to the present example is preferably administered at a rate of one 0.1ml (1000 IU) spray per nostril daily starting just prior to cold and flu season.

The stabilised tablets of the present example comprise the following components:

	4kg Batch
<u>Hydrogel core</u>	
Polyvinylpyrrolidone/Acetic acid ethenyl polymer 3.508kg	
<u>Coating Liquid</u>	
Interferon alpha 40 million IU	
Mannitol	0.200kg
Propylene glycol	0.150kg
Gelatin (succinylated)	0.050kg
Glycine	0.012kg
Albumin	0.001kg
Ascorbic Acid	0.006kg
standard sodium phosphate buffer to pH 7	0.073kg
Purified water to	2.000kg

The stabilised tablets of the present example were prepared according to the invention using the following steps:

1. Interferon alpha, glycine, mannitol, gelatin succinylated, propylene glycol, ascorbic acid and buffers are dissolves in water for injection then filtered through 0.02 micron membrane filter. Add albumin and make up to weight with water for injection.
2. Huttlin Turbojet chamber sterilised by heat treatment at 180°C as instructed by the equipment manufacturer.
3. Apparatus switched to circulating filtered nitrogen mode.
4. Hydrogel core loaded into the Huttlin Turbojet chamber via vacuum and heated up to 60°C for one hour.
5. Hydrogel core product temperature reduced to 40°C.
6. Contents of the apparatus chamber fluidised at a rate of 300 cubic metres per hour.
7. Interferon alpha coating turbojet sprayed onto the hydrogel core under saturated moisture conditions at a rate of 25g/minute.
8. Resultant product dried to less than 2% moisture content.
9. Microcapsules compressed, according to standard procedures in the art, into 200mg tablets.
10. Tablets packaged into nitrogen flushed aluminium/aluminium foil packs and stored at a temperature not exceeding 25°C.

Each 200mg tablets produced according to this example contains 50,000 IU interferon alpha.

The constituents of the liquid diluent formulation of the present example are provided in table 10 below.

Table 10

	% w/w
GELATIN BP/Eur.P	0.10
POVIDONE BP/Eur.P	0.10
DISODIUM EDETATE BP/Eur.P	0.14
POLYSORBATE 80 BP/Eur.P	0.20
DEXTRAN 45,000 BP/Eur.P	0.22
SODIUM DIHYDROGEN PHOSPHATE BP/Eur.P (Anhydrous Weight)	0.27
DISODIUM HYDROGEN PHOSPHATE BP/Eur.P (Anhydrous Weight)	0.58
GLYCINE BP/Eur.P	0.10
SODIUM PROPYL HYDROXYBENZOATE BP/Eur.P	0.03
SODIUM METHYL HYDROXYBENZOATE BP/Eur.P	0.09
ALBUMIN BPC	0.05
TOTAL	2.93
PURIFIED WATER BP/Eur.P to	100%

Example 4e - Stabilized Interferon Alpha Nasal Spray for treatment of cold, flu and other respiratory diseases.

The present example, provides an alternative diluent formulation containing additional active ingredients that may help relieve cold and flu symptoms.

The consumer product of the present example contains the two components listed in Example 4d; the tablet containing the interferon alpha and the diluent within a spray applicator bottle or the like. Similarly, the reconstituted interferon nasal spray will contain 1000 IU interferon alpha /0.1 ml.

The preferred dosage regime of the present example is: One 0.1ml (1000 IU) spray per nostril morning and night when mild flu or cold symptoms appears. Continue treatment for 5 to 10 days.

The tablet component of the present example is prepared according to process of Example 2d.

The diluent formula of the present formulation is given in Table 11 below:

Table 11 % w/w

OXYMETAZOLIN HYDROCHLORIDE USP	0.05
DEXCHLORPHENIRAMINE MELEATE USP	1.00
GELATIN BP/Eur.P	0.10
POVIDONE BP/Eur.P	0.10
DISODIUM EDETATE BP/Eur.P	0.14
POLYSORBATE 80 BP/Eur.P	0.20
DEXTRAN 45,000 BP/Eur.P	0.22
SODIUM DIHYDROGEN PHOSPHATE BP / Eur.P (Anhydrous Weight)	0.27
DISODIUM HYDROGEN PHOSPHATE BP/Eur.P (Anhydrous Weight)	0.58
GLYCINE BP/Eur.P	0.10
SODIUM PROPYL HYDROXYBENZOATE BP/Eur.P	0.03
SODIUM METHYL HYDROXYBENZOATE BP/Eur.P	0.09
ALBUMIN BPC	0.05
TOTAL	2.93
PURIFIED WATER BP/Eur.P to	100%

Example 4f - Stabilized Interferon Alpha Eye Wash or Drops for prevention of red eye disease

As previously discussed, correctly formulated aqueous interferon alpha may be stable up to one month, but not two, at room temperature. Accordingly, interferon eye washes or drops, in a ready to use liquid form, with a viable commercial shelf life (of approximately 12 to 18 months for example) are unavailable.

A viable alternative is to have the principal active ingredient (interferon alpha) presented as a pre-constituted room temperature stable tablet. Accordingly, just prior to use the interferon alpha tablet may be added to an eye wash, or eye drop, bottle containing an acceptable liquid diluent. The reconstituted solution would have a shelf life of around 4 weeks at room temperature. The user may discard the bottle at the completion of treatment.

The consumer product of the present example contains the two components listed in Example 4d; the tablet containing the interferon alpha and the diluent (albeit in an appropriate eye wash or drop bottle, in this example). Similarly, the reconstituted interferon eye wash or drops contain 1000 IU interferon alpha /0.1 ml, with 0.1ml being the preferred single administration dose.

The preferred dosage regime of this example is: One 0.1ml (1000 IU) spray/drop per eye, twice daily for treatment of red eye.

The interferon alpha tablet formulae of the present example is formulated and processed according to that described in Example 4d above.

The diluent formulation of the present example is provided in Table 12 below. It will be appreciated that this liquid diluent is preferably autoclaved, or otherwise sterilised.

Table 12 % w/w

POVIDONE BP/Eur.P	0.10
DISODIUM EDETATE BP/Eur.P	0.14
POLYSORBATE 80 BP/Eur.P	0.20
DEXTRAN 45,000 BP/Eur.P	0.22
SODIUM DIHYDROGEN PHOSPHATE BP / Eur.P (Anhydrous Weight)	0.27
DISODIUM HYDROGEN PHOSPHATE BP/Eur.P (Anhydrous Weight)	0.58
GLYCINE BP/Eur.P	0.10
SODIUM PROPYL HYDROXYBENZOATE BP/Eur.P	0.03
SODIUM METHYL HYDROXYBENZOATE BP/Eur.P	0.09
ALBUMIN BPC	0.05
TOTAL	2.93
PURIFIED WATER BP/Eur.P to	100%

Example 4g - Stabilized Interferon Skin Spray – same formulation as eyewash

A skin spray, for wound healing, for example, was formulated according to Example 4f. However, in this example, the liquid diluent was presented in an appropriate container for delivery to the skin.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, and compositions referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

Particularly, it will be appreciated by those of general skill in the art to which the present invention relates, that while the present invention has been described and exemplified with

reference to the preparation of specific proteins and micro-organisms it is equally applicable to the preparation of any cells and/or proteins or peptides of interest. For example, the process of the invention may be readily applicable to the preparation of hormones, including growth hormones, and growth factors, EPO, Calcitonin, Human Growth Hormone, Gamma Interferon, Interleukins, Insulin, IL 2, CSF, GM-CSF, interferons, calcitonin, enzymes such as streptokinase, muramidase, pancrease, amylase, protease, lypase, cellulase, bromelaine, papain and the like.

The specification provides examples of preferred dosage rates for the use of a number of the novel formulations made according to the invention. Alternative dosages and concentrations of active therein are envisaged by the inventors and those of general skill in the art to which the invention relates will readily be able to formulate products, according to the present invention, which have alternative concentrations of active therein.

Further, it will be appreciated that a product according to the invention may be manipulated or further formulated in order to arrive at a desired dose form. For example, the microcapsules of the invention may be encapsulated to form standard capsule unit doses, or may be combined with various standard excipients and diluents used in the art, to form tablets or liquid formulations, for example. Those of skill in the art will appreciate many other ways in which the micro-capsules of the invention may be further formulated and that they are contemplated by the inventors of the present invention.

Finally, it is contemplated by the inventors of the present invention that the novel process described herein may be used to prepare other materials and to manipulate materials to particular ends. In particular, it is envisaged that the inventive process may be manipulated to allow for enteric coating of microcapsules using solvents or aqueous methods such as Sodium salts of Cellulose Acetate Phthalate, to create sustained release properties by changing the core material polymers to a higher molecular weight or by using a combination of hydrogel core such as high molecular weight gelatin, polyvinyl pyrrolidone, alginates, carboxymethyl cellulose, various cellulose derivative, polyethylene glycols, albumin, karregreenin (one of ordinary skill in the art of formulation will be able

to provide various combinations to create a desired release profile), create time release properties by varying the nature of polymers used and the thickness of the coatings and to allow for micro-distribution of trace materials among large amount of solid mass.

Titles, headings, or the like are provided to enhance the reader's comprehension of this document, and should not be read as limiting the scope of the present invention.

The entire disclosures of all applications, patents and publications, cited above and below, if any, are hereby incorporated by reference.

The reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that that prior art forms part of the common general knowledge in the field of endeavour.

Throughout this specification, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

DATED this TWENTY-FIFTH day of JANUARY 2001

~~**Thomas Ko Sai Ying**~~ Gainful Plan Limited

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ABSTRACT

A method of preparing biological materials, particularly biological proteins, and materials produced from said method, are described. The method generally comprises at least the steps: (i) providing a coating liquid comprising at least one active, a sugar polymer and a water soluble/miscible solvent; (ii) providing a quantity of micro-particles comprising at least water soluble gel forming solid particles; (iii) fluidising said quantity of micro-particles within a processing chamber of a suitable apparatus to form a fluidised bed of said micro-particles; (iv) spraying said coating liquid onto said fluidised bed from beneath the fluidised bed to coat said micro-particles therewith under saturated moisture conditions; and (v) allowing coated micro-particles to dry.